REMARKS

Reconsideration and allowance of the above-referenced application are respectfully requested.

Claim 12 has been cancelled, and claims 1 and 8 have been amended. In particular, it is believed that the amendment to claim 8 overcomes the Examiner's objection relating thereto.

Additionally, Applicants attorney appreciates the Examiner's acknowledgement that several of the Section 102(b) and Section 103(a) rejections have been withdrawn.

Rejection of Claims 1-7 Under 35 U.S.C. 112, Second Paragraph

The Examiner has rejected claims 1-7 under Section 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In response, Applicants submit that the Examiner's concerns, giving rise to the rejection, have been adequately addressed by the amendments to claim 1 shown above. (Additionally, the Examiner is respectfully requested to review Example VIII of the application that

discusses how the combination assay is carried out and the generation of only one signal.)

In view of the above, it is submitted that the Section 112, second paragraph rejection of claims 1-7 has been overcome and should be withdrawn accordingly.

Rejection of Claims 3 and 10 Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 3-10 under Section 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner's Position

The Examiner requests that Applicant point out where each of the cell lines 107-35-54 (referred to as H35C54), 110-81-17, 13-975-157 and 14-1350-210 and corresponding ATCC numbers are disclosed in U.S. Patent No. 5,753,430. The Examiner contends that it is not readily apparent that the designations and deposited material of the issued patent are the same as the cell lines recited in the claims and specification of the instant application.

The Applicants' Position

In response, Applicants submit that the deposit relating to hybridoma cell line 107-35-53 (also referred to as H35C54) is described in col. 2, lines 54-55 of U.S. Patent No. 5,753,430. The deposit relating to cell line 110-81-17 (also referred to as H81C17) is described in col. 2, lines 52-53 of U.S. Patent No. 5,753,430. Further, the deposit relating to cell line 13-975-157 is described in col. 2, lines 61-62 of U.S. Patent No. 5,753,430, and the deposit relating to cell line 14-1350-210 is described in col. 2, lines 64-66 of U.S. Patent No. 5,753,430.

In view of the above, it is submitted that the rejection of claims 3 and 10 under Section 112, first paragraph has been overcome and should be withdrawn accordingly.

Rejection of Claims 13 and 14 Under 35 U.S.C. 102(e)

The Examiner has rejected claims 13 and 14 under Section 102(e) as being anticipated by U.S. Patent Publication No. 2002/0192639 A1 (Chien et al.)

The Examiner's Position

The Examiner contends that Chien et al. disclose kits comprising an HCV antigen and an HCV antibody coated on a single solid phase and conjugates comprising a signal-

generating compound, anticipating the claimed subject matter.

The Applicants' Position

Applicants respectfully submit that, as evidenced by the attached Rule 131 Declaration, the invention claimed in claims 13 and 14 was conceived of and reduced to practice prior to the filing date of the Chien et al. patent publication (i.e., June 14, 2001). Thus, the Section 102(e) rejection has been overcome and should be withdrawn accordingly.

Rejection of Claims 13 and 14 Under 35 U.S.C. 102(e)

The Examiner has rejected claim 13 and 14 under Section 102(e) as being anticipated by U.S. Patent Publication No. 2003/0049608 A1 (Bahl et al.).

The Examiner's Position

The Examiner contends that Bahl et al. disclose kits comprising an HCV antigen and an HCV antibody coated on a single solid phase and conjugates comprising a signal-generating compound, anticipating the claimed subject matter.

The Applicants' Position

Applicants respectfully submit that, as evidenced by the attached Rule 131 Declaration, the invention claimed in claims 13 and 14 was conceived of and reduced to practice

prior to the filing date of the Bahl et al. patent publication (i.e., March 28, 2002). Thus, the Section 102(e) rejection has been overcome and should be withdrawn accordingly.

Rejection of Claims 13 and 14 Under 35 U.S.C. 102(e) or 35 U.S.C. 103(a)

The Examiner has rejected claims 13 and 14 under Section 102(e) as being anticipated by or, in the alternative, under Section 103(a) as obvious over U.S. Patent Publication No. 2002/0173493 A1 (Aoyagi et al.).

The Examiner's Position

The Examiner contends that the composition of Aoyagi comprising a container containing an HCV antigen and an HCV antibody coated on a solid phase and a conjugate comprising a signal-generating compound is believed to anticipated the subject matter of claims 13 and 14, although not explicitly referred to as a kit, but if not, it would have been obvious to package the composition in the form of a kit as is conventionally done for reasons of convenience and economy.

The Applicants' Position

Applicants respectfully submit that, as evidenced by the attached Rule 131 Declaration, the invention claimed in

claims 13 and 14 was conceived of and reduced to practice prior to the filing date of the Aoyagi et al. patent publication (i.e., April 26, 2002). Thus, the Section 102(e) rejection has been overcome and should be withdrawn accordingly.

Further, due to the information presented in the Rule 131 Declaration related to conception and reduction to practice prior to the filing date of the Aoyagi et al. document, it is submitted that the Declaration also overcomes the alternative Section 103(a) rejection, as the document cannot serve as a basis for the rejection. Thus, the Section 103(a) rejection has also been overcome and should be withdrawn accordingly.

In view of the above, it is submitted that the rejection of claims 13 and 14 under Section 102(e) as anticipated by or, in the alternative, under Section 103(a) as obvious over Aoyagi have been overcome and should therefore be withdrawn.

Rejection of Claims 8-11 and 15 Under 35 U.S.C. 103(a)

The Examiner has rejected claims 8-11 and 15 under Section 103(a) as being unpatentable over Aoyagi et al.

The Examiner's Position

The Examiner contends that the method of Aoyagi et al. differs from the claimed method only by exemplifying the use of an enzyme label in place of a chemiluminescent label. Thus, the Examiner alleges that it would have been obvious to one of ordinary skill in the art, based on the teachings of Aoyagi, to have used a chemiluminescent label because Aoyogi teaches that any conventional label may be used.

The Applicants' Position

Due to the information presented in the Rule 131 Declaration related to conception and reduction to practice of the subject matter of claims 8-11 and 15, prior to the filing date of the Aoyagi et al. document, it is submitted that the Aoyagi et al. document must be eliminated as a basis for a Section 103(a) rejection. Thus, the cited Section 103(a) rejection has been overcome and should be withdrawn accordingly.

Rejection of Claims 8-11 and 15 Under 35 U.S.C. 103(a)

The Examiner has rejected claims 8-11 and 15 under Section 103(a) as being unpatentable over Chien et al.

The Examiner's Position

The Examiner alleges that it would have been obvious to one of ordinary skill in the art, based on the teachings of Chien, to have detected both HCV antigen and antibody simultaneously, using a single solid phase coated with HCV antibody and HCV antigen and antibody-chemiluminescent compound conjugates to a generate a detectable signal since Chien exemplifies an assay using the same format as claimed and suggests the use of a chemiluminescent label.

The Applicants' Position

It is submitted that the information presented in the Rule 131 Declaration related to conception and reduction to practice of the subject matter of claims 8-11 and 15, prior to the filing date of the Chien et al. document, establishes that this document must also be eliminated as a basis for a Section 103(a) rejection. Thus, the cited Section 103(a) rejection has been overcome and should be withdrawn accordingly.

Rejection of Claims 8-12, 14 and 15 (now claims 8-11, 14 and 15) Under 35 U.S.C. 103(a)

The Examiner has rejected claims 8-12, 14 and 15 (now claims 8-11, 14 and 15, due to the cancellation of claim

12) under Section 103(a) as being unpatentable over Bahl et al. in view of Chien et al.

The Examiner's Position

The Examiner contends that it would have been obvious to one of ordinary skill in the art to have substituted a chemiluminescent label as taught by Chien for the exemplified enzyme of Bahl et al. because Bahl et al. requires only a "detectable label" and because Chien teaches that any conventional label, including a chemiluminescent label, can be used in an HCV antigen-antibody combination assay.

The Applicants' Position

As noted above, the information presented in the Rule 131 Declaration related to conception and reduction to practice of the subject matter of claims 8-12, 14 and 15, prior to the filing dates of both Bahl et al. and Chien et al. documents, eliminates both documents as a basis for a Section 103(a) rejection. Thus, the cited Section 103(a) rejection has been overcome and should be withdrawn accordingly.

Rejection of Claims 12, 14 and 15 (now claims 14 and 15) under 35 U.S.C. 103(a)

The Examiner has rejected claims 12, 14 and 15 (now claims 14 and 15, due to the cancellation of claim 12)

under Section 103(a) as being unpatentable over Dawson et al. in view of Masalova et al.

The Examiner's Position

The Examiner contends that Dawson et al. disclose co-detection of HCV core antigen and HCV antibodies in a chemiluminescent assay but do not specifically disclose a solid-phase immunoassay format, the use of HCV core monoclonal antibodies, or kits. Additionally, the Examiner asserts that Masalova et al. disclose the use of a solid-phase immunoassay format for sandwich immunoassays. Thus, the Examiner alleges that, while Dawson nor Masalova specifically disclose kits, it would have been obvious to one of ordinary skill in the art, at the time the invention was made, to package two solid phase components to be used together in the form of a kit for reasons of convenience and economy and that such components are necessarily kept in containers.

The Applicants' Position

The Applicants respectfully traverse the rejection of claims 12, 14 and 15 (now claims 14 and 15) under Section 103(a) as being obvious over Dawson et al. in view of Masalova et al.

It is submitted that Dawson et al. disclose the "co-detection" of HCV antibodies and HCV antigens; however,

Dawson et al. certainly do not disclose or suggest the use an HCV antigen and an HCV antibody, both coated on one solid phase, in a simultaneous detection method or single assay system designed to detect both HCV antigen and HCV antibody in a test sample. Further, Dawson et al. do not disclose the use of an additional component which is a conjugate comprising a signal-generating compound attached to an antibody (see present claim 14), wherein the signal-generating compound may be acridinium (see present claim 15), as in the claimed invention. Rather, Dawson et al. disclose the detection of both HCV antigens and HCV antibodies, by use of separate assays, after seroconversion has occurred.

Further, as noted previously, it is submitted that the Masalova et al. reference does not remedy the deficiencies present in Dawson et al. In particular, Masalova et al. disclose the detection of HCV core protein using a monoclonal antibody sandwich enzyme immunoassay. Masalova et al., however, do not disclose or suggest an immunoassay involving detection of both HCV antigen and HCV antibody by use of an HCV antigen and HCV antibody, both attached to the solid phase, as well as a conjugate, in a single assay system.

In view of the above, it is submitted that the Section 103(a) rejection of claims 14 and 15 over Dawson et al. in view of Masalova et al. has been overcome. One of ordinary skill in the art certainly would not have been motivated to have created the claimed invention based upon the teachings or suggestions of Dawson et al., either alone or in combination with Masalova et al. The claimed invention is not rendered obvious, and the rejection should therefore be withdrawn.

In conclusion, it is believed that the subject application is in condition of allowance and Notice to that effect is respectfully requested.

Should any questions arise concerning the above, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,
D. Shah, et al.

23492

ABBOTT LABORATORIES
Telephone: (847) 935-1729
Facsimile: (847) 938-2623

Cheryl L. Becker
Registration No. 35,441
Attorney for Applicants

MARKED UP VERSION OF CLAIMS SHOWING CHANGES MADE

IN THE CLAIMS:

Please amend claims 1, 8 and 12 as follows:

1. (amended) A method of simultaneously detecting at least one Hepatitis C Virus (HCV) antigen and at least one HCV antibody in a test sample comprising the steps of:

(a) contacting said test sample with [a mixture of]:

1) at least one HCV antigen or portion thereof coated on a solid phase, for a time and under conditions sufficient for the formation of antibody/antigen complexes[, presence of said antibody/antigen complexes indicating presence of said at least one HCV antibody in said test sample]; and

2) at least one antibody to HCV or portion thereof coated on said solid phase, to which said at least one HCV antigen or portion thereof is also coated, for a time and under conditions sufficient for the formation of antigen/antibody complexes[,]; and

(b) detecting the presence of said antibody/antigen complexes, presence of said antibody/antigen complexes indicating presence of said at least one HCV antibody in said test sample and detecting presence of said antigen/antibody complexes, presence of said antigen/antibody complexes indicating presence of said at least one HCV antigen in said test sample.

8. (twice amended) A method for simultaneously detecting the presence of at least one HCV antigen and at least one HCV antibody in a test sample comprising the steps of:

[a)] (a) contacting said test sample with: 1) at least one HCV antigen or portion thereof coated on a solid phase, for a time and under conditions sufficient for the formation of antibody/antigen complexes and 2) at least one HCV antibody or portion thereof coated on said solid phase, for a time and under conditions sufficient for the formation of antigen/antibody complexes;

[b)] (b) adding a conjugate to the resulting antibody/antigen complexes of (a)(1) for a time and under conditions sufficient to allow said conjugate to bind to the bound antibody in (a)(1), wherein said conjugate comprises a second antibody attached to a chemiluminescent compound capable of generating a detectable signal; and simultaneously adding a second conjugate to the resulting antigen/antibody complexes of (a)(2) for a time and under conditions sufficient to allow said conjugate to bind to the bound antigen in (a)(2), wherein said conjugate comprises a third antibody attached to said chemiluminescent compound capable of generating a detectable signal; and

[c)] (c) detecting [said] a single generated signal, presence of said signal indicating presence of said at least one HCV antigen, at least one HCV antibody, or both, in said test sample.

14. (twice amended) The kit of [claim 12 or] claim 13 further comprising at least one conjugate comprising a signal-generating compound attached to an antibody.

#25 Decl. w/ exhibits 8.4.03

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shah et al.

Serial No.: 09/891,983

Filed: June 26, 2001

For: METHODS FOR THE SIMULTANEOUS DETECTION OF HCV ANTIGENS AND HCV ANTIBODIES

Case No.: 6821.US.01

Examiner: Wortman, D.

Group Art Unit: 1648

Certificate of Mailing under 37 CFR §1.8(a): I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Date of Deposit: Kimberly A. Iorio 7-25-03
Kimberly A. Iorio

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, GEORGE J. DAWSON and LILY JIANG, citizens of the United States of America and residents of Libertyville, Illinois and Mundelein, Illinois, respectively, do declare and say that:

We are co-inventors of the above-referenced application for patent filed on June 26, 2001.

In the Office Action of April 29, 2003, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Chien et al. (U.S. Patent Publication No. 2002/0192639 A1). Additionally, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Bahl et al.

(U.S. Patent Publication No. 2003/0049608 A1). Further, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Additionally, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Further, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chien et al. (U.S. Patent Publication No. 2002/0192639 A1). Also, claims 8-12, 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bahl et al. (U.S. Patent Publication No. 2003/0049608 A1) in view of Chien et al. (U.S. Patent Publication No. 2002/0192639 A1).

We, along with our co-inventors, conceived and reduced to practice, the invention claimed in claims 13 and 14 prior to the filing date of Chien et al. (i.e., June 14, 2001), prior to the filing date of Bahl et al. (i.e., March 28, 2002) and prior to the filing date of Aoyagi et al. (i.e., April 26, 2002). Further, we, along with our co-inventors, conceived and reduced to practice the invention claimed in claims 8-11 and 15 prior to the filing date of Aoyagi et al. (i.e., April 26, 2002) and prior to the filing date of Chien et al. (i.e., June 14, 2001).

Additionally, we, along with our co-inventors, conceived and reduced to practice the invention claimed in claims 8-12, 14 and 15 prior to the filing date of Bahl et al. (March 28, 2002) as well as Chien et al. (i.e., June 14, 2001). These assertions are evidenced by the following:

Attached Exhibit A illustrates that, prior to June 14, 2001 (i.e., the filing date of Chien et al. and the earliest filing date of the documents cited above), we, along with our co-inventors, developed a method for the simultaneous detection of HCV antigens and HCV antibodies in a test sample. In particular, as evidenced by Exhibit A, in one embodiment, the HCV antigens were to be captured on a solid phase, and then the captured antigens were be detected with an antibody (e.g., monoclonal antibody) labeled with a reporter molecule. Further, the solid phase was to be coated with various HCV proteins (e.g., NS3, NS4 and fragments of the core protein) in order to capture HCV antibodies. The antibodies would then be recognized by a second antibody (e.g., goat anti-human IgG) labeled with a reporter molecule.

Further, Exhibit A also illustrates a schematic view of the assay. In particular, the figure establishes how the antibodies in the test sample are to be detected as

well as how the core antigens are to be detected using conjugated monoclonal antibodies.

Exhibit B illustrates that prior to the June 14, 2001 filing date of Chien et al., we, along with our co-inventors, carried out the assay and obtained positive data. In particular, Exhibit B illustrates various reagents used in the assay (i.e., those coated on the solid phase) and evidences that upon running the assay, results were obtained indicating that one could detect HCV antigen and HCV antibody simultaneously in a sample.

In summary, the attached Exhibits establish that the claimed invention was conceived of and reduced to practice, prior to the filing date of Chien et al. (i.e., June 14, 2001) as well as the subsequent filing dates of Bahl et al. and Aoyagi et al.

Although all the dates on Exhibits A and B have been blocked out, such dates are prior to June 14, 2001.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Respectfully submitted,

By: _____
George U. Dawson

Date: 07/14/03

By: _____
Jily Jiang

Date: 07/14/03

09/891,983 #25

EXHIBIT A

ABBOTT LABORATORIES
RESEARCH DEPARTMENT

BOOK NO. 61,959

PROJECT HCV antigen test

EXP. OR CODE NO.

There have been recent indications that HCV core proteins can be detected in serums of HCV infected individuals, most notably the publications from Tonen Corporation (Tanaka et al, Journal of Hepatology 1995 23: 742-745: and Aoyagi et al, in the Journal of Clinical Microbiology 1999 37:1802-1808. There have been no published disclosures pertaining to an antigen/antibody combo test for detection of exposure to HCV to date. There are several possible methods for devising a combo HCV test, allowing detection of both antibodies and antigens associated with exposure to HCV. Current antigenic targets for the antibody test include recognition of viral proteins derived from several different open reading frames of the virus including core + envelope proteins as well as proteins from nonstructural regions designated as NS (nonstructural) 2, NS_3, NS_4 and NS5. Commercialized tests currently utilize HCV proteins from NS_3, NS_4 and/or NS5.

(Continued on page 5)

SIGNATURE Dawn Jones DATE

WITNESSED BY Lily Ruiz DATE

PROJECT HCV antigen test

EXP. OR CODE NO.

A potential combo test would continue to utilize one or more virally derived proteins from HCV for antibody detection but would also employ antibodies generated against HCV proteins to develop an antigen sandwich assay which captures HCV proteins on a solid phase (microcellulose, microparticles, polystyrene plate or beads) and then further detects the captured protein with a labeled antibody. One of the most likely targets for detection of HCV antigens is the HCV core protein. While Toray corporation has clearly demonstrated utility of an HCV antigen test, there has been no clear indication of a combo test being developed. For this reason, the following proposal is made - Abbott Labs would develop an antibody/antigen combo test, allowing simultaneous detection of antibodies & antigens associated with exposure to HCV. In one example of this combo assay a solid phase would be coated with HCV proteins (NS3, NS4 and fragments of the HCV core protein) and also coated with antibodies to HCV. (continued on page 6)

SIGNATURE [signature] DATE

WITNESSED BY [signature] DATE

ABBOTT LABORATORIES
RESEARCH DEPARTMENT

PROJECT HCV antigen test

EXP. OR CODE NO.

This solid phase would capture antibodies to HCV or would also capture HCV proteins (e.g. Core protein). The captured antibodies (that were captured due to antibodies binding to HCV proteins) would be recognized by a second antibody (e.g. goat anti-human IgG) that is labeled with a reporter molecule (horseradish peroxidase, acridinium, biotin etc). allowing detection of antibodies directed agst the solid phase-bound proteins derived from HCV.

The captured antigens would be recognized in one example of the combo assay by specific antibodies (e.g. monoclonal antibodies) against the core protein. this specific antibody would be labeled with a reporter molecule (horseradish peroxidase BIOTIN, acridinium) to allow detection of the Bound antigen.

One of the important differences between the combo test for HCV and HIV as proposed here is that one continues to be able to detect antibodies to core and core antigen at the same time. In order to do this successfully, the core protein needs to be re-engineered. See page 7

SIGNATURE _____ DATE _____

WITNESSED BY _____ DATE _____

PROJECT HCV antigen test

EXP. OR CODE NO.

The core protein of HCV consists of 191 amino acids. For detection of antibodies to HCV only segments of the core molecule would be needed. For example it is known that there are epitope associated with antibody detection at amino acids 9–88 based on 11 tetrapeptide peptides. Thus, in one version of a combo assay the solid phase would be coated with – NS3, NS4 and/or NS5 proteins and a modified core protein (containing needed epitopes) as well as one or more monoclonal antibodies (or possibly polyclonal antibodies to core). See Figure on page 7. Further the conjugates would recognize the bound antibodies (captured with specific antigen) or bound antigens (captured with specific antibodies). The candidate core proteins would be: recombinant core proteins (aa 1–100, aa 1–120, aa 8–89, aa 9–99 etc) with monoclonal antibodies recognizing epitopes outside of the sequences recognized by antibodies in human serum. Alternatively one could use peptides 6 mers or greater covering major epitopes between amino acids 1–100. Further, the antigens on the solid phase could be re-engineered to include amino acid substitutions, deletions, etc.

SIGNATURE _____ DATE _____

WITNESSED BY _____ DATE _____

ABBOTT LABORATORIES
RESEARCH DEPARTMENT

PROJECT Schematic for Combo Ag

BOOK NO. 61,959

Schematic of Potential HCV Combo Antibody/Antigen Test

Liquid Phase Conjugates:

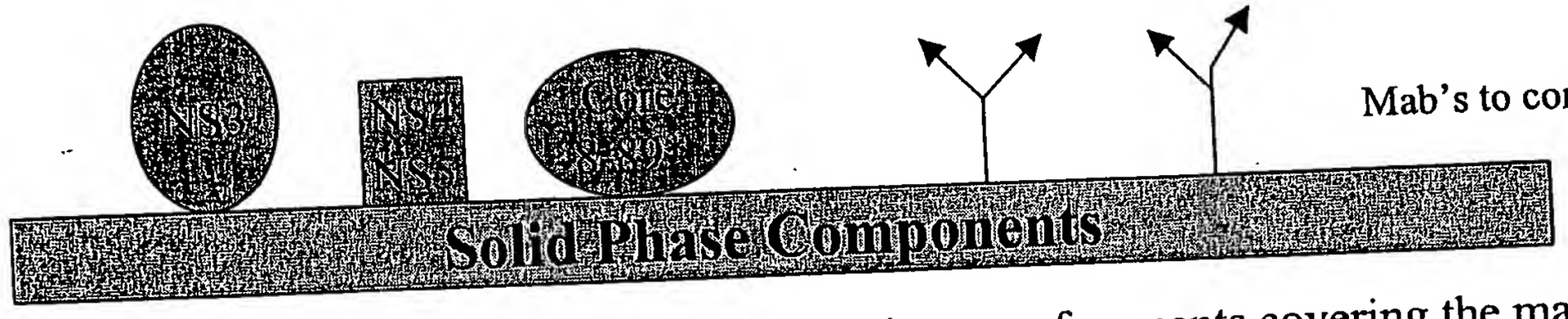
Goat anti-human conjugate
- detect antibodies to core, NS3-5

Conjugated mab's to core
- detects core antigen in serum
- conjugates must not recognize solid phase core

Mab's agst aa:

1-8, 89-190, 8-89

(amino acid deletions substitutions are made in core solid phase proteins/peptides)



Mab's to core 1-8, 89-190

*Recombinant core aa 8-89 may appear as a single entity or as fragments covering the major epitopes…sequences may be modified by deletions, substitutions between aa 8-89 that do not perturb major epitopes. Synthetic peptides covering aa 8-89 would also be a viable alternative.

SIGNATURE [signature] DATE

WITNESSED BY [signature] DATE

09/89/98

#2-5

EXHIBIT B

8

ABBOTT LABORATORIES
RESEARCH DEPARTMENT

BOOK NO. 68,160

PROJECT HCV combo Assay
Ab, Ag
EXP. OR CODE NO. Blended ags and conj.

This is the first demonstration of a combination antibody/Ag test for HCV.

N/A 08/31/00 21:29:16 L JIANG

Blended Up and Blended conjugate

Up: HC31 (DF=3 Coating conc: 200ug/ml) + C11-14 (0.09% 0.4um)
Conjugate: C11-10 (100ng/ml 1:16) + 6A52B (1/5dilution In HIV combo CD)
Washes: HIV ag transfer wash Dev lot 5/ final wash : HCv Ag prep
SDB: 6A52Q
Up diluent: 18498 HCv Ab assay up diluent
S/A configuration: HCV

			Combo Assay		Ab Assay	
SubA	SubB	Mean counts	P/N	Mean Counts 08/28/00	P/N
1502	1923	1712.5	2.17	33952	8.64
808	852	780		3930.17	
755	745				
719		719	0.91	4618.75	1.23
1157	1063	1110	1.41	36800.67	9.36
9785	9035	8410	11.91	147307.5	37.48
7972	8237	8104.5	10.28		
2550	3639	3094.5	3.92		
5227	5280	5258.5	6.66		
842	899	870.5	1.10	1427	0.36
1954	1773	1863.5	2.36	2059.5	0.52
2552	2463	2507.5	3.17	1704.5	0.43
3606	3507	3556.5	4.50	1716.5	0.44
2882	3120	3001	3.80	1507.5	0.38
2055	2280	2172.5	2.75	1671.5	0.43
	3707	3707	4.69	1665	0.42

3.17

Samples	P/N
PC (Ab)	55.82
NC (Ab)	88.36
99800	8.64
Panel A	33.48
E2 1/20 dil	59.56
Promed 9992161	64.19
PC JV 016929	51.04
PC JV017220	36.11
Sero-Tec panel #3	61.14
4	
5	
6	
7	
8	
9	

A
6
1023
V COMBO ASSAY

1277

21511

Ag Assay
Mean counts
08/24/00

79

4409.83
6980.5
681.5
2645
4706.5
5071
4258
2853
4829

cont. on pg # 10

As the first Blend up and Conj. results are encouraging. Dilute Conj more for Next run.

SIGNATURE _____ DATE _____

WITNESSED BY _____ DATE _____

ABBOTT BORATORIES
RESEARCH DEPARTMENT

PROJECT HCV combo Assay

EXP. OR CODE NO. Cont. from page #8.

DESCRIPTION OF PANEL MEMBERS -

NC - negative control - pooled plasma individually screened as negative for HCV antibodies by a commercialized assay- **Code: 6A52E. Prism HCv Ab Assay Negative Calibrator.**
PC - positive control - pooled anti-HCV positive plasma diluted in negative control . **Code: 6A52F. Prism HCV Ab Assay Positive Calibrator.**

99800 - Plasma(human) Recalcified Negative Bulk.

Panel A - an anti-HCV positive plasma that has been diluted in negative control to provide a mid range sample to cutoff in the PRISM antibody assay.

E2 1/20 - an anti-HCV positive sample that has been diluted in negative control - the E2 antibody panel was utilized to titrate the potency of HCV E2 antigen coated microparticles

Promed 9992161 - an antibody positive sample obtained from ProMeDx (Plainville, MA)

PC JV 16929 - Sero-Tec HCV RNA positive human plasma .
PC P JV17220 - Sero-Tec HCV RNA positive human plasma .

SeraTec Panel members 3-9 - serial bleeds obtained from a plasma donor identified at SeraTec as being anti-HCv negative and HCV antigen positive.

A panel of specimens previously characterized as having antibodies to HCV or being negative for antibodies to HCV but positive for HCV RNA and HCV antigens were tested in a preliminary HCV combination antibody.antigen test.

Reagents utilized in combo test
Microparticles specific for HCV antigen detection (up's coated with C11-14 as described on RB: 67093 page 100) and microparticles specific for HCV antibody detection (up's coated with HCV recombinant protein HC 31 as described on RB: 68160page 2) were blended to produce a solid phase that would allow simultaneous detection of HCV antibodies and HCV antigens in a single reaction well. (The blended microparticles contained 0.19% solids, representing a mixture of 0.09% up's coated with C11-14 and 0.1% coated with HC31). The conjugates were also a mixture of two separate acridinium labeled proteins. Acridinium labeled C11-10 was utilized for HCV antigen detection (recognizing HCV antigens captured on the C11-14 microparticles) and an acridinium labeled monoclonal antibodies against biotin -labeled gaot anti-human IgG (presented as a pre-complex - see RB: 52226m301) was utilzied to detect human anti-HCV IgG bound to the HC-31 coated microparticles.

Results

The panel described above was run on 3 different PRISM-based assays. One of the assays detected HCV antibodies, a second test detected HCV antigens and a third test (the combo assay) detected both HCV antibodies and HCV antigens.
Sampels have a positive to negative ratio (P/N) ratio of 3.0 or greater were considered positive.
The data presented in the table on RB68160page 8 indicate that the combo assay allows detection both of antibody positive samples (e.g. panel E2 1/20, ProMed 9992161, PC JV 016929 and PC JV 17220) and HCV antigen positive samples (Sera Tec panel members 5-9). Thus, this single combo assay performed in a single reaction well detects most of the samples that were positive in two separately performed assays, the HCV antibody test and the HCV antigen test. This is the first demonstration of a combo HCV antibody / HCV antigen test at Abbott Laboratories, and is the first example of the HCV antibody /antigen combo test ideas presented in Redbook 61,959: pages 1-8. Other iterations of the HCV combo test will be presented over the next several weeks/months.

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PROJECT HCV combo Assay. *

EXP. OR CODE NO.

Title: HCV combo Assay: Blended up and Blended conjugate

Purpose: To blend the HCV core peptide coated ups, NS3NS4 coated up, c11-14 coated ups together and c11-10 , aHigG Acr* conjugate together for HCV combo first demonstration.

Materials and Samples
RB: 68160001 and 68160011.

(Core peptide Ag + NS3NS4 for Ab Detection)
c11-14 mAb coated up for Ag Detection)

Preparation:
Add Avidin 11-28 (df = 20) and NS3NS4 (df = 10) and c11-14 (0.09% seradyn)
Add conjugate c11-10 (50ng/ml) and aHigG Acr* (10ng/ml)

Results:

HCV Combo (11-28, NS3NS4,c11-14 c11-10, aHigG) 9 12

Conclusions:
The combo assay successfully detected all the Ab pos. samples and Ag positive samples.

Next Steps:
Dilute the AhIgG conjugate to 7ng/ml and 2 ng/ml

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HCV COMBO 11-28,NS3NS4
C11-14 C11-10 AHIGG

HCV Combo Assay

Blended ups: HCv Core Bio-11-28(Df=20)+ NS3NS4 HCV Ag (DF=10)+ C11-14(0.09%)
Conjugate: c11-10(5ong/ml) + aHIgG Acr*(10ng/ml)
Washes: HCV Ag Assay Transfer: HIV Ag Devlot5, Final wash: HCV Ag final wash prep 8/1/2000
SDB: HCV Ab (6A52Q)
S/A (1023) configuration: HCV

	SubA	SubB	Mean	P/N
PC (Ab)	3454	3656	3555	4.84
PC (Ag)	5303	6014	5658.5	7.71
PC(Ag)	4288	3722	4005	5.46
NC(99800)	637	831	734	
E2 1/20	12480	13092	12786	17.42
ProMed 9990196	11449			15.60
9990164	15	No conjugate was added		
9990162	10060		10060	13.71
9990212	13925		13925	18.97
Sero-Tec panels #3	956		956	1.30
4	2347		2347	3.20
5	3400		3400	4.63
6	4673		4673	6.37
7	4265		4265	5.81
8	3045		3045	4.15

*Materials: Code/List/Desc/Lot see RB: 68160

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EXP. OR CODE NO. Cont. from page #17

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ups 11-28+NS3NS4+C11-14 — Conjugate 50ng/ml + 7ng/ml

d1-10 vH1/a* 57051

	SubA	SubB	Mean	P/N
NC	452	456	454.00	
E2 (1/20)	7753	7804	7778.50	17.13
PC (Ag)	4462	4407	4434.50	9.77
9990212	7611		7611.	16.76
9996196	6878		6878	15.15
9996164	5133		5133	11.31
Sero-Tec panel #3	1257		1257	2.77
4	2640		2640	5.81
5	2870		2870	6.32
6	4917		4917	10.83
BBI HCv sero 907 #1	5595		5595	12.32
2	2707		2707	5.96
3	2614		2614	5.76
4	2701		2701	5.95
5	2343		2343	5.16
6	4443		4443	9.79
7	8147		8147	17.94

ups 11-28+NS3NS4+C11-14 — Conjugate 50ng/ml + 2ng/ml

	SubA	SubB	Mean	2ng/ml P/N	7ng/ml P/N
NC	277	246	261.50		
E2 (1/20)	2831	2879	2855.00	10.92	17.13
PC (Ag)	4213	4099	4156.00	15.89	9.77
9990212	2773		2773	10.60	16.76
9996196	2249		2249	8.60	15.15
9996164	1918		1918	7.33	11.31
Sero-Tec panel #3	927		927	3.54	2.77
4	2299		2299	8.79	5.81
5	3002		3002	11.48	6.32
6	5112		5112	19.55	10.83
BBI HCv sero 907 #1	3754		3754	14.36	12.32
2	3363		3363	12.86	5.96
3	2230		2230	8.53	5.76
4	2404		2404	9 19	5.95
5	1743		1743	6.67	5.16
6	2084		2084	7.97	9.79
7	1566		1566	5.99	17.94

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BOSTON BIOMEDICA, INC.

Anti-HCV Seroconversion Panel (PHV907)

HCV Genotype 1A

Panel Member	Bleed Date	Day No.	ABBOTT 3.0 HCV S/CO	ORTHO HCV 3.0 S/CO	Combo data Conj @7 ng/ml S/N	Combo data Conjug @ 2 ng/ml S/N	Abbott Ag Only Test S/CO (1)	Roche Amplicor RNA copies/ml
PHV907-1		0	0.1	0.0	12.3	14.4	25.68	3 x 10e6
PHV907-2		4	0.1	0.0	6.0	12.9	20.41	2 x 10e6
PHV907-3		7	0.1	0.0	5.8	6.5	17.88	1 x 10e6
PHV907-4		13	0.2	0.1	6.0	9.2	15.98	1 x 10e6
PHV907-5		18	0.8	0.5	5.2	6.7	6.88	1 x 10e6
PHV907-6		21	1.4	1.0	9.8	8.0	7.90	1 x 10e6
PHV907-7		164	>5.0	>5.0	18.0	6.0	0.70	nd

Data above demonstrates on seven member seroconversion panel, that HCV RNA and HCV Antigens can be detected from the first bleed date (through the sixth bleed date, but the seventh bleed date is negative for HCV antigen. The antibody tests Ortho 3.0 and Abbott 3.0 failed to detect antibodies in the first five bleed dates (through)...

The combo test detected exposure to HCV for all seven bleed dates.

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BOOK NO. 68,160

PROJECT PRISM HCV Ag/Ab combo

EXP. OR CODE NO. HCV combo Assay Random Donor Population

Reagents: Same as Exp# 68160017.

09/18/00 11:00:17 L JIANG

HCV Combo Assay Population

		SubA	SubB	Mean	P/N	Cutoff(3nc)	S/CO	Cutoff (2.5nc)	S/CO	Cut off (2.33nc)	S/CO
		3737	3854	3795.5	15.30	744	5.10	620	6.57	577.84	6.12
		2949	2785	2867	11.66		3.85		4.96		4.62
		214	282	248							
		182					0.24		0.31		0.29
		163					0.22		0.28		0.26
		292					0.39		0.51		0.47
		172					0.23		0.30		0.28
		265					0.36		0.46		0.43
		172					0.23		0.30		0.28
		271					0.36		0.47		0.44
		329					0.44		0.57		0.53
		200					0.27		0.35		0.32
		247					0.33		0.43		0.40
		196					0.26		0.34		0.32
		246					0.33		0.43		0.40
		432					0.58		0.75		0.70
		214					0.29		0.37		0.35
		161					0.22		0.28		0.26
		215					0.29		0.37		0.35
		140					0.19		0.24		0.23
		227					0.31		0.39		0.37
		284					0.38		0.49		0.46
		209					0.28		0.36		0.34
		200					0.27		0.35		0.32
		185					0.25		0.32		0.30
		248					0.33		0.43		0.40
		209					0.28		0.36		0.34
		291					0.39		0.50		0.47
		241					0.32		0.42		0.39
1	PC (Ag)										
2	E2 (1/20)										
3	NC (99800)										
4	GCRBC plasma #1										
5											
6											
7											
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